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=> d his

     (FILE 'HOME' ENTERED AT 14:37:00 ON 09 APR 2003)

     FILE 'BIOSIS, CAPLUS, MEDLINE, EMBASE, SCISEARCH' ENTERED AT 14:37:24 ON
     09 APR 2003
L1          280 S CALDOTENAX
L2           65 S L1 AND (DNA POLYMERASE)
L3           10 S L2 AND (MUTANT OR VARIANT)
L4            6 DUP REM L3 (4 DUPLICATES REMOVED)
L5         1160 S MYCOBACTERIOPHAGE
L6           12 S L5 AND (DNA POLYMERASE)
L7            0 S L6 AND (MUTANT OR VARIANT)
L8            8 DUP REM L6 (4 DUPLICATES REMOVED)
L9         7102 S (THERMUS THERMOPHILUS)
L10         483 S L9 AND (DNA POLYMERASE)
L11          42 S L10 AND (MUTANT OR VARIANT)
L12          32 DUP REM L11 (10 DUPLICATES REMOVED)
L13           0 S L12 AND 669
L14           0 S L12 AND (F-669 OR F669 OR PHE669 OR PHE-669)
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=> d l4 ibib ab 1-13

L4 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:658653 CAPLUS
DOCUMENT NUMBER: 137:197516
TITLE: Hybrid **DNA polymerase** with high fidelity by reducing 5'-3' exonuclease activity and increasing 3'.fwdarw.5' exonuclease activity and preparation and use thereof
INVENTOR(S): Chatterjee, Deb K.
PATENT ASSIGNEE(S): Invitrogen Corporation, USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002119461	A1	20020829	US 2001-902741	20010712
PRIORITY APPLN. INFO.:			US 2000-217738P P	20000712

AB The invention relates to a DNA and RNA polymerases with high fidelity by substitution **variants** or fusion proteins with increased 3'.fwdarw.5' exonuclease activity and high fidelity and uses. The invention relates to a DNA and RNA polymerases which have increased fidelity (or reduced misincorporation rate). In particular, the invention relates to a method of making such polymerases by increasing or enhancing 3'-5' exonuclease activity of a polymerase by, for example, substituting the 3'-5' exonuclease domain of one polymerase with a 3'-5' exonuclease domain with the desired activity from another polymerase. The invention also relates to DNA mols. contg. the genes encoding the polymerases of the invention, to host cells contg. such DNA mols. and to methods to make the polymerases using such host cells. The polymerases of the invention are particularly suited for nucleic acid synthesis, sequencing, amplification and cDNA synthesis. Specifically, various hybrid Taq **DNA polymerases** are prepd. by swapping various 5'-3'-exonuclease domain, 3'-5'-exonuclease, and polymerase domain from natural and **mutant** polymerase sources. The activities are characterized for TneA **DNA polymerase mutant** (with D137A and D323A mutations, deficient in the 5-3' exonuclease and 3'-5' exonuclease activities), TneB (with D137A mutation, deficient in the 5'-3' exonuclease activity), Chi polymerase (Taq/Tne chimeric **DNA polymerase**). These polymerase domains can be selected from the group consisting of Tne DNApolymerase, Taq **DNA polymerase**, Tma **DNA polymerase**, Tth **DNA polymerase**, Tli, VENTTM DNApolymerase, Pfu DNApolymerase, DEEPVENTTM, **DNA polymerase**, Pwo **DNA polymerase**, Bst **DNA polymerase**, Bca **DNA polymerase**, Tfl **DNA polymerase**, and **mutants**, **variants** and derivs. thereof.

L4 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:101324 CAPLUS
DOCUMENT NUMBER: 134:159466
TITLE: Nuclease amino acid sequence **variants** without 5'.fwdarw.3'-exonuclease activity and their use in genotyping assays
INVENTOR(S): Sayers, Jon; Patel, Dipak
PATENT ASSIGNEE(S): University of Sheffield, UK
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001009343	A1	20010208	WO 2000-GB2915	20000802
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1212430	A1	20020612	EP 2000-949725	20000802
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				

PRIORITY APPLN. INFO.: GB 1999-18150 A 19990803
WO 2000-GB2915 W 20000802

AB The invention relates to exonuclease **variants** that lack detectable 5'.fwdarw.3' exonuclease activity but retain endonuclease activity. The invention also relates to vectors encoding the **variant** exonucleases, the recombinant prodn. of same, and use of the **variants** in genotyping assays. The genotyping assays involve comparing the DNA fragments obtained after denaturing SDS-polyacrylamide gel electrophoresis of DNA substrates that were cleaved by the nuclease **variant**(s). The genotyping assays measure differences in secondary nucleic acid structure, caused by nucleic acid sequence variation in the test substrates, which affect nuclease activity. Structure-specific binding and endonucleolytic cleavage of a pseudo-Y substrate were measured for phage T5 gene D15 exonuclease K83R and Taq **DNA polymerase** K82R **variants**. Phage T5 D15 exonuclease K83R and Taq polymerase K82R enzymes were used to identify alleles of the human IL-1.beta.-511 polymorphism. This invention also provides a kit, with std. DNA controls, for genotyping assays.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 13 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998224649 EMBASE
TITLE: Lethality of high linear energy transfer cosmic radiation to Escherichia coli DNA repair-deficient **mutants** during the 'SL-J/FMPT' space experiment.
AUTHOR: Harada K.; Nagaoka S.; Mohri M.; Ohnishi T.; Sugahara T.
CORPORATE SOURCE: K. Harada, 1-3-2-207 Oji, Oji-cho, Kitakatsuragi-gun, Nara 636-0002, Japan. kazuki@os.gulf.or.jp
SOURCE: FEMS Microbiology Letters, (1 Jul 1998) 164/1 (39-45). Refs: 24
ISSN: 0378-1097 CODEN: FMLED7
PUBLISHER IDENT.: S 0378-1097(98)00184-0
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We investigated the lethal and mutagenic effects of high linear energy transfer cosmic radiation on 11 strains of Escherichia coli, including DNA repair-deficient **mutants**, using the Radiation Monitoring Container and Dosimeter in the space shuttle 'Endeavour' as part of the 'SL-J/FMPT' space experiment, the 'Fuwatto '92' project. After the return to earth of the shuttle, we evaluated survival and mutations of samples in

space and matched controls. The surviving fractions were determined by means of colony count on broth agar plates, and the mutation frequencies were estimated by appearance of arg+ revertants on minimal agar plates. The average of the total equivalent dose rate during this space flight was 0.202 mSv/day as measured by the plastic radiation detectors and the thermoluminescent dosimeters in the Radiation Monitoring Container and Dosimeter. The combined action of **DNA polymerase** and 3' .fwdarw. 5' exonuclease activities was found to make the greatest contribution to the repair of cosmic radiation-induced DNA damage, 5' .fwdarw. 3' exonuclease and recombination repair enzyme activities made a moderate contribution, whereas UV endonuclease activity was not involved in this DNA repair process.

L4 ANSWER 4 OF 13 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96191402 EMBASE
DOCUMENT NUMBER: 1996191402
TITLE: Changes in cellular proteins of **Deinococcus radiodurans** following .gamma.- irradiation.
AUTHOR: Tanaka A.; Hirano H.; Kikuchi M.; Kitayama S.; Watanabe H.
CORPORATE SOURCE: Biotechnology Laboratory, TRCRE,Gunma, Japan
SOURCE: Radiation and Environmental Biophysics, (1996) 35/2 (95-99).
ISSN: 0301-634X CODEN: REBPAT
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
014 Radiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In order to examine radiation-induced proteins in an extremely radioresistant bacterium, **Deinococcus radiodurans** R1, changes in cellular proteins after .gamma.-irradiation were analysed by two-dimensional gel electrophoresis and silver staining. Nine proteins (190, 120, 87, 60, 58, 52, 46, 41 and 41 kDa) were increased (or appeared) and more than 13 proteins diminished after .gamma.-irradiation at 6 kGy. Increase of eight proteins (except for 190-kDa protein) was prevented when the cells were irradiated in the presence of chloramphenicol. Three proteins, 87, 60 and 46 kDa, continued to be synthesized during post-irradiation incubation, and the amounts of these proteins increased with higher doses in a range of 1-12 kGy. Changes in the amount of proteins after irradiation in the R1 strain were compared with those in a moderately radioresistant **mutant** (rec 1) and in a highly radiosensitive **mutant** (rec30). These three proteins were increased in both R1 and rec 1, but not in rec 30, suggesting that they are characteristic for radioresistant strains. In addition, from the microsequence analysis, the 46- kDa protein was found to be homologous to the EF-Tu protein of Escherichia coli, whereas the remarkable homologous sequence to the N-terminal of the 60- kDa protein was not found among the known proteins.

L4 ANSWER 5 OF 13 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 95394840 MEDLINE
DOCUMENT NUMBER: 95394840 PubMed ID: 7665511
TITLE: Genetic characterization of forty ionizing radiation-sensitive strains of **Deinococcus radiodurans**: linkage information from transformation.
AUTHOR: Mattimore V; Udupa K S; Berne G A; Battista J R
CORPORATE SOURCE: Department of Microbiology, Louisiana State University, Baton Rouge 70803, USA.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Sep) 177 (18) 5232-7.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951020
Last Updated on STN: 19951020
Entered Medline: 19951012

AB Natural transformation was used to help define a collection of ionizing radiation-sensitive strains of **Deinococcus radiodurans**. Three putative rec mutations were identified, as were three pol alleles. Forty of the ionizing radiation-sensitive strains were placed into 16 linkage groups, and evidence obtained indicates that each linkage group consists of a cluster of mutations not more than 1,000 bp apart. In addition, a new class of D. radiodurans **mutant** was described that, although radioresistant, appears to recover from ionizing radiation-induced DNA damage slowly relative to other strains of D. radiodurans.

L4 ANSWER 6 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:193451 BIOSIS
DOCUMENT NUMBER: PREV199698749580
TITLE: The amino acid sequence required for 5' fwdarw 3' exonuclease activity of Bacillus caldotenax **DNA polymerase**.
AUTHOR(S): Ishino, Yoshizumi (1); Takahashi-Fujiii, Asako; Uemori, Takashi; Imamura, Mitsuo; Kato, Ikunoshin; Doi, Hirofumi
CORPORATE SOURCE: (1) Biotechnol. Res. Lab., Takara Shuzo, Otsu, Shiga 520-21 Japan
SOURCE: Protein Engineering, (1995) Vol. 8, No. 11, pp. 1171-1175. ISSN: 0269-2139.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We studied the 5' fwdarw 3' exonuclease activity of Bacillus caldotenax **DNA polymerase** by site-directed mutagenesis. Among seven **mutants** constructed, two **mutant DNA polymerases** with an amino acid substitution of Gly184 fwdarw Asp or Gly192 fwdarw Asp were confirmed to be deficient in this exonuclease. The two positions corresponded to those of the Escherichia coli **DNA polymerase** I **mutants** defective in 5' fwdarw 3' exonuclease, polA480ex and polA214. These results provide experimental support for the proposed amino acid sequence essential for the 5' fwdarw 3' exonuclease activity associated with eubacterial polymerase I-like **DNA polymerases** (family A), including E. coli and Thermus aquaticus.

L4 ANSWER 7 OF 13 TOXCENTER COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:438680 TOXCENTER
DOCUMENT NUMBER: EMIC-94774
TITLE: Defective transformation of chromosomal markers in **DNA polymerase** I **mutants** of the radioresistant bacterium **Deinococcus radiodurans**.
AUTHOR(S): Fuchs P; Agostini H; Minton K W
CORPORATE SOURCE: Department of Pathology, F.E. Haebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.
CONTRACT NUMBER: GM39933 (GM)
SOURCE: Mutation Research, (1994 Sep 1) 309 (2) 175-84. Journal Code: NNA. ISSN: 0027-5107.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: EMIC
OTHER SOURCE: EMIC MED-94352335
LANGUAGE: English
ENTRY DATE: Entered STN: 20021200
Last Updated on STN: 20021200

AB The transformation efficiency of six independently selected chromosomal markers (four for rifampicin resistance and two for acriflavine resistance) was found to be reduced by about 3 logs in a **Deinococcus radiodurans** strain that was isogenic with wild type except for an insertional mutation in the pol gene that eliminated **DNA polymerase** I activity (strain 6R1A). D. radiodurans strains UV17 and 303, previously obtained by chemical mutagenesis, were determined to be partially deficient in DNA Pol I activity as assessed in a permeabilized cell system. Both UV17 and 303 demonstrated intermediate transforming efficiencies that correlated with their levels of residual polymerase activity. The transformation efficiency of strain 6R1A could be greatly restored by expression of cloned E. coli DNA Pol I, but not to wild-type levels. Plasmid transfer and chromosomal duplication insertion were not substantially affected by lack of DNA Pol I activity. D. radiodurans is known to possess extraordinarily efficient repair pathways for DNA damage, and is refractory to DNA damage-induced mutagenesis caused by numerous agents, including several that cause base mispairing. We suggest that D. radiodurans may differ from other naturally transformable bacteria in that DNA Pol I is needed to efficiently convert most drug-resistance markers. This unusual mechanism may be required to accomplish chromosomal conversion prior to correction of donor DNA by this organism's efficient repair pathways.

L4 ANSWER 8 OF 13 TOXCENTER COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:436615 TOXCENTER
DOCUMENT NUMBER: EMIC-92665
TITLE: Restoration of the DNA damage resistance of **Deinococcus radiodurans DNA polymerase mutants** by Escherichia coli **DNA polymerase** I and Klenow fragment.
AUTHOR(S): Gutman P D; Fuchs P; Minton K W
CORPORATE SOURCE: Department of Pathology, F. E. Haebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.
CONTRACT NUMBER: GM39933 (GM)
SOURCE: Mutation Research, (1994 Jan) 314 (1) 87-97. Journal Code: NNA. ISSN: 0027-5107.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: EMIC
OTHER SOURCE: EMIC MED-94067196
LANGUAGE: English
ENTRY DATE: Entered STN: 20021200
Last Updated on STN: 20021200

AB **Deinococcus radiodurans** and other species of this genus share extreme resistance to ionizing radiation and many other agents that damage DNA. D. radiodurans **mutant** strains defective in a deinococcal **DNA polymerase** that is homologous with E. coli **DNA polymerase** I are highly sensitive to DNA damage. In the current work we have inquired whether E. coli DNA Pol I can substitute for D. radiodurans Pol in partially or fully restoring to pol- D. radiodurans **mutants** the extreme DNA damage-resistance typical of this organism. The E. coli polA gene or a 5'-truncated polA gene that encodes the Klenow fragment were introduced and expressed in two different D. radiodurans pol- **mutants**: Strain 303, which is a chemically mutagenized derivative, and strain 6R1A, which is isogenic with wild-type D. radiodurans except for an insertional mutation within the pol gene. Expression of E. coli polA in both of these **mutants** fully restored wild-type resistance to ionizing- and UV254-radiation and mitomycin-C exposure. Expression of the Klenow fragment-encoding gene restored wild-type resistance to D. radiodurans strain 303, but only partial resistance to strain 6R1A. The observation that E. coli DNA Pol I is as effective as D. radiodurans Pol in restoring damage resistance, indicates that D. radiodurans DNA Pol per se does not have special

properties that are essential or prerequisite for expression of the extreme resistance of D. radiodurans.

L4 ANSWER 9 OF 13 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 1994:571884 CAPLUS
DOCUMENT NUMBER: 121:171884
TITLE: Defective transformation of chromosomal markers in **DNA polymerase** I **mutants** of the radio-resistant bacterium **Deinococcus radiodurans**
AUTHOR(S): Fuchs, Pinhas; Agostini, Heidi; Minton, Kenneth W.
CORPORATE SOURCE: Department of Pathology, F.E. Hebert School of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD, 20814-4799, USA
SOURCE: Mutation Research (1994), 309(2), 175-84
CODEN: MUREAV; ISSN: 0027-5107
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The transformation efficiency of six independently selected chromosomal markers (four for rifampicin resistance and two for acriflavine resistance) was found to be reduced by about 3 logs in a **Deinococcus radiodurans** strain that was isogenic with wild type except for an insertional mutation in the pol gene that eliminated **DNA polymerase** I activity (strain 6R1A). D. radiodurans strains UV17 and 303, previously obtained by chem. mutagenesis, were detd. to be partially deficient in DNA Pol I activity as assessed in a permeabilized cell system. Both UV17 and 303 demonstrated intermediate transforming efficiencies that correlated with their levels of residual polymerase activity. The transformation efficiency of strain 6R1A could be greatly restored by expression of cloned E. coli DNA Pol I, but not to wild-type levels. Plasmid transfer and chromosomal duplication insertion were not substantially affected by lack of DNA Pol I activity. D. radiodurans is known to possess extraordinarily efficient repair pathways for DNA damage, and is refractory to DNA damage-induced mutagenesis caused by numerous agents, including several that cause base mispairing. We suggest that D. radiodurans may differ from other naturally transformable bacteria in that DNA Pol I is needed to efficiently convert most drug-resistance markers. This unusual mechanism may be required to accomplish chromosomal conversion prior to correction of donor DNA by this organism's efficient repair pathways.

L4 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 1994:72469 CAPLUS
DOCUMENT NUMBER: 120:72469
TITLE: Restoration of the DNA damage resistance of **Deinococcus radiodurans DNA polymerase mutants** by Escherichia coli **DNA polymerase** I and Klenow fragment
AUTHOR(S): Gutman, Pablo D.; Fuchs, Pinhas; Minton, Kenneth W.
CORPORATE SOURCE: F. E. Hebert Sch. Med., Univ. Health Sci., Bethesda, MD, 20814-4799, USA
SOURCE: Mutation Research (1994), 314(1), 87-97
CODEN: MUREAV; ISSN: 0027-5107
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Deinococcus radiodurans** and other species of this genus share extreme resistance to ionizing radiation and many other agents that damage DNA. D. radiodurans **mutant** strains defective in a deinococcal **DNA polymerase** that is homologous with E. coli **DNA polymerase** I are highly sensitive to DNA damage. In the current work, the authors have inquired whether E. coli DNA Pol I can substitute for D. radiodurans Pol in partially or fully

restoring to pol- D. radiodurans **mutants** the extreme DNA damage-resistance typical of this organism. The E. coli polA gene or a 5'-truncated polA gene that encodes the Klenow fragment was introduced and expressed in two different D. radiodurans pol- **mutants**: Strain 303, which is a chem. mutagenized deriv., and strain 6R1A, which is isogenic with wild-type D. radiodurans except for an insertional mutation within the pol gene. Expression of E. coli polA in both of these **mutants** fully restored wild-type resistance to ionizing- and UV254-radiation and mitomycin-C exposure. Expression of the Klenow fragment-encoding gene restored wild-type resistance to D. radiodurans strain 303, but only partial resistance to strain 6R1A. The observation that E. coli DNA Pol I is as effective as D. radiodurans Pol in restoring damage resistance, indicates that D. radiodurans DNA Pol per se does not have special properties that are essential or prerequisite for expression of the extreme resistance of D. radiodurans.

L4 ANSWER 11 OF 13 TOXCENTER COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:434950 TOXCENTER
DOCUMENT NUMBER: EMIC-90947
TITLE: Identification, sequencing, and targeted mutagenesis of a **DNA polymerase** gene required for the extreme radioresistance of **Deinococcus radiodurans**.
AUTHOR(S): Gutman P D; Fuchs P; Ouyang L; Minton K W
CORPORATE SOURCE: Department of Pathology, F. E. Haebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799.
CONTRACT NUMBER: GM39933 (GM)
SOURCE: Journal of Bacteriology, (1993 Jun) 175 (11) 3581-90. Journal Code: HH3. ISSN: 0021-9193.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: EMIC
OTHER SOURCE: EMIC MED-93273728
LANGUAGE: English
ENTRY DATE: Entered STN: 20021200
Last Updated on STN: 20021200

AB **Deinococcus radiodurans** and other species of the same genus share extreme resistance to ionizing radiation and many other agents that damage DNA. Two different DNA damage-sensitive strains generated by chemical mutagenesis were found to be defective in a gene that has extended DNA and protein sequence homology with polA of Escherichia coli. Both **mutant** strains lacked **DNA polymerase**, as measured in activity gels. Transformation of this gene from wild-type D. radiodurans restored to the **mutants** both polymerase activity and DNA damage resistance. A technique for targeted insertional mutagenesis in D. radiodurans is presented. This technique was employed to construct a pol **mutant** isogenic with the wild type (the first example of targeted mutagenesis in this eubacterial family). This insertional **mutant** lacked **DNA polymerase** activity and was even more sensitive to DNA damage than the **mutants** derived by chemical mutagenesis. In the case of ionizing radiation, the survival of the wild type after receiving 1 Mrad was 100% while survival of the insertional **mutant** extrapolated to 10(-24). These results demonstrate that the gene described here encodes a **DNA polymerase** and that defects in this pol gene cause a dramatic loss of resistance of D. radiodurans to DNA damage.

L4 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 1993:532676 CAPLUS
DOCUMENT NUMBER: 119:132676
TITLE: Identification, sequencing, and targeted mutagenesis of a **DNA polymerase** gene required for the extreme radioresistance of **Deinococcus radiodurans**

AUTHOR(S): Gutman, Pablo D.; Fuchs, Pinhas; Ouyang, Ling; Minton, Kenneth W.
CORPORATE SOURCE: F. E. Hebert Sch. Med., Uniformed Serv. Univ. Health Sci., Bethesda, MD, 20814-4799, USA
SOURCE: Journal of Bacteriology (1993), 175(11), 3581-90
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Deinococcus radiodurans** and other species of the same genus share extreme resistance to ionizing radiation and many other agents the damage DNA. Two different DNA damage-sensitive strains generated by chem. mutagenesis were found to be defective in a gene that has extended DNA and protein sequence homol. with polA of Escherichia coli. Both **mutant** strains lacked **DNA polymerase**, as measured in activity gels. Transformation of this gene from wild-type D. radiodurans restored to the **mutants** both polymerase activity and DNA damage resistance. A technique for targeted insertional mutagenesis in D. radiodurans is presented. This technique was employed to construct a pol **mutant** isogenic with the wild type (the first example of targeted mutagenesis in this eubacterial family). This insertional **mutant** lacked **DNA polymerase** activity and was even more sensitive to DNA damage than the **mutants** derived by chem. mutagenesis. In the case of ionizing radiation, the survival of the wild type after receiving 1 Mrad was 100% while survival of the insertional **mutant** extrapolated to 10-24. These results demonstrate that the gene described here encodes a **DNA polymerase** and that defects in this pol gene cause a dramatic loss of resistance of D. radiodurans to DNA damage.

L4 ANSWER 13 OF 13 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 80185850 EMBASE
DOCUMENT NUMBER: 1980185850
TITLE: DNA-membrane complex restoration in Micrococcus radiodurans after X-irradiation: Relation to repair, DNA synthesis and DNA degradation.
AUTHOR: Dardalhon-Samsonoff M.; Averbeck D.
CORPORATE SOURCE: Sect. Biol., Inst. Curie, 75231 Paris Cedex 05, France
SOURCE: International Journal of Radiation Biology, (1980) 38/1 (31-52).
CODEN: IJRBA3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal
FILE SEGMENT: 014 Radiology
023 Nuclear Medicine
022 Human Genetics
004 Microbiology
LANGUAGE: English

AB The DNA-membrane complex in Micrococcus radiodurans was shown to be essentially constituted of proteins, lipids and DNA. The complex was dissociated immediately after X-irradiation of cells and restored during post-incubation in complete medium. In X-irradiated protoplasts some DNA remained associated with the complex. Restoration of the complex during post-incubation was only seen in a medium favouring **DNA polymerase** and ligase activities. Under this condition no DNA synthesis occurred, suggesting that complex restoration may involve ligase activity. The complex restoration in the wild type and the X-ray sensitive **mutant** UV17 of M. radiodurans was strictly dependent on the X-ray dose. It was correlated with survival and DNA degradation but always preceded the onset of DNA synthesis after X-irradiation. At the same dose the complex restoration was about 2-fold lower in **mutant** than in wild type cells indicating that the restoration of the complex is related to repair capacity. The results are consistent with the idea that the complex protects X-irradiated DNA of M. radiodurans from further breakdown and, subsequently, permits DNA synthesis and repair to occur.

=> d l4 ibib ab 1-4

L4 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:51298 CAPLUS
DOCUMENT NUMBER: 136:97304
TITLE: **DNA polymerase** substitution **variants** or fusion proteins with increased 3'.fwdarw.5' exonuclease activity and high fidelity and uses
INVENTOR(S): Chatterjee, Deb K.
PATENT ASSIGNEE(S): Invitrogen Corporation, USA
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

Post dated

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002004022	A1	20020117	WO 2001-US21790	20010711
WO 2002004022	C2	20020117		
WO 2002004022	C1	20030227		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2002018749	A5	20020121	AU 2002-18749	20010711

PRIORITY APPLN. INFO.:
US 2000-217738P P 20000712
WO 2001-US21790 W 20010711

AB The present invention relates to substantially pure polymerases having high fidelity. Specifically, the polymerases of the present invention are polymerases (e.g., **DNA polymerases** or RNA polymerases) which have been modified to increase the fidelity of the polymerase (compared to the unmodified or unmutated polymerase), thereby providing a polymerase which has a lower misincorporation rate (reduced misincorporation). Preferably, the polymerases of the invention are thermostable or mesophilic polymerases. The present invention also relates to cloning and expression of the polymerases of the invention, to DNA mols. contg. the cloned gene, and to hosts which express said genes. The polymerases of the present invention may be used in DNA sequencing, amplification reactions, nucleic acid synthesis and cDNA synthesis. Such polymerases are modified in their 3'-5' exonuclease domain such that the fidelity of the enzyme is increased or enhanced. Modifications can include mutations in the 3'-5' exonuclease domain which result in increased 3'-5' exonuclease activity, or partial or complete substitution of the 3'-5' exonuclease domain with a 3'-5' exonuclease domain from a polymerase having increased 3'-5' exonuclease activity. In the present invention, we have made hybrid Taq polymerase where the inactive 3'-5'-exonuclease domain of Taq polymerase was replaced with an active 3'-5'-exonuclease domain from another thermostable **DNA polymerase**. We have shown that the hybrid Taq polymerase displayed all three activities, 5'-3'-exonuclease activity, 3'-5'-exonuclease activity and the polymerase activity suggesting that the domain shuffling did not impair the structural integrity. We have also shown that both proofreading activity and the polymerase act in concert indicating that the hybrid polymerase is acting like a true high-fidelity polymerase. Therefore, the hybrid polymerase will be extremely 'useful for PCR or other applications. The present invention relates in

particular to **mutant** Poll type **DNA polymerase** (preferably thermostable **DNA polymerases**) wherein one or more amino acid changes have been made in the 3'-5' exonuclease domain which renders the enzyme more faithful (higher fidelity) in nucleic acid synthesis, sequencing and amplification. In accordance with the invention, other functional changes may be made to the polymerases having increased fidelity. For example, the polymerase may also be modified to reduce 5' exonuclease activity, and/or reduce discrimination against ddNTP's.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:658653 CAPLUS
DOCUMENT NUMBER: 137:197516
TITLE: Hybrid **DNA polymerase** with high fidelity by reducing 5'-3' exonuclease activity and increasing 3'.fwdarw.5' exonuclease activity and preparation and use thereof
INVENTOR(S): Chatterjee, Deb K.
PATENT ASSIGNEE(S): Invitrogen Corporation, USA
SOURCE: U.S. Pat. Appl. Publ., 20 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002119461	A1	20020829	US 2001-902741	20010712
PRIORITY APPLN. INFO.:			US 2000-217738P P	20000712

AB The invention relates to a DNA and RNA polymerases with high fidelity by substitution **variants** or fusion proteins with increased 3'.fwdarw.5' exonuclease activity and high fidelity and uses. The invention relates to a DNA and RNA polymerases which have increased fidelity (or reduced misincorporation rate). In particular, the invention relates to a method of making such polymerases by increasing or enhancing 3'-5' exonuclease activity of a polymerase by, for example, substituting the 3'-5' exonuclease domain of one polymerase with a 3'-5' exonuclease domain with the desired activity from another polymerase. The invention also relates to DNA mols. contg. the genes encoding the polymerases of the invention, to host cells contg. such DNA mols. and to methods to make the polymerases using such host cells. The polymerases of the invention are particularly suited for nucleic acid synthesis, sequencing, amplification and cDNA synthesis. Specifically, various hybrid Taq **DNA polymerases** are prepd. by swapping various 5'-3'-exonuclease domain, 3'-5'-exonuclease, and polymerase domain from natural and **mutant** polymerase sources. The activities are characterized for TneA **DNA polymerase mutant** (with D137A and D323A mutations, deficient in the 5-3' exonuclease and 3'-5' exonuclease activities), TneB (with D137A mutation, deficient in the 5'-3' exonuclease activity), Chi polymerase (Taq/Tne chimeric **DNA polymerase**). These polymerase domains can be selected from the group consisting of Tne DNApolymerase, Taq **DNA polymerase**, Tma **DNA polymerase**, Tth **DNA polymerase**, Tli, VENTTM DNApolymerase, Pfu DNApolymerase, DEEPVENTTM, **DNA polymerase**, Pwo **DNA polymerase**, Bst **DNA polymerase**, Bca **DNA polymerase**, Tfl **DNA polymerase**, and **mutants**, **variants** and derivs. thereof.

L4 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:255210 CAPLUS
DOCUMENT NUMBER: 134:292060
TITLE: Cold-sensitive **mutant DNA polymerases** for improved use in PCR
INVENTOR(S): Barnes, Wayne M.; Kermekchiev, Milko B.
PATENT ASSIGNEE(S): Washington University, USA
SOURCE: U.S., 22 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6214557	B1	20010410	US 2000-587856	20000606
US 6316202	B1	20011113	US 2001-777537	20010206
US 2002004203	A1	20020110		
US 6333159	B1	20011225	US 2001-777538	20010206
WO 2001094562	A2	20011213	WO 2001-US18236	20010605
WO 2001094562	A3	20020530		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1287146	A2	20030305	EP 2001-944300	20010605
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 2000-587856 A3 20000606
WO 2001-US18236 W 20010605

AB Provided are **mutant DNA polymerases** having at least one mutation which exhibit substantially reduced polymerase activity at 25.degree. when compared to the same **DNA polymerases** without the at least one mutation and which exhibit normal or near-normal polymerase activity at optimum temps. when compared to the same **DNA polymerases** without the at least one mutation. Also provided are amino acid sequences and nucleic acid sequences encoding such **DNA polymerases**, and vector plasmids and host cells suitable for the expression of these sequences. Also described herein are improved methods for performing polymerase chain reaction (PCR) amplification and other genetic manipulations and analyses using the **mutant DNA polymerases** of the invention. Thus, the Thermus aquaticus **DNA polymerase** with a deletion of the N-terminal 235 amino acids (Klentaq-235) and with the triple mutations E626K/Q690R/I707L produces sharp bands under either cold start or hot start PCR conditions.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:193940 CAPLUS
DOCUMENT NUMBER: 130:219872
TITLE: Analogs of thermostable **DNA polymerases** with broadened substrate specificities for DNA sequencing with dye-labeled nucleotides
INVENTOR(S): Gelfand, David H.; Kalman, Lisa Vivian; Myers, Thomas W.; Reichert, Fred Lawrence; Sigua, Christopher Lim
PATENT ASSIGNEE(S): F. Hoffmann-La Roche AG, Switz.

SOURCE: Eur. Pat. Appl., 31 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 902035	A2	19990317	EP 1998-116786	19980905
EP 902035	A3	19990623		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2243985	AA	19990311	CA 1998-2243985	19980903
US 6346379	B1	20020212	US 1998-146631	19980903
NO 9804157	A	19990312	NO 1998-4157	19980910
AU 9884161	A1	19990325	AU 1998-84161	19980910
AU 741366	B2	20011129		
BR 9803419	A	20000208	BR 1998-3419	19980910
JP 11137284	A2	19990525	JP 1998-258414	19980911
CN 1218832	A	19990609	CN 1998-124526	19980911
US 2002142333	A1	20021003	US 2002-52417	20020117

PRIORITY APPLN. INFO.: US 1997-58525P P 19970911
US 1998-146631 A1 19980903

AB Modified thermostable **DNA polymerases** that incorporate unconventional nucleotides such as those labeled with fluorescein family dyes with greater efficiency are described. These polymerases are advantageous in many in vitro DNA synthesis applications, such as DNA sequencing, synthesis of labeled DNA and the prodn. of labeled primer extension products, esp. in chain termination DNA sequencing. Genes for these polymerase and expression vectors for their manuf. are described. The enzyme may be used in DNA sequencing kits where it will provide significant advantages in cost and efficiency. Analogs of the Taq polymerase were generated by mutagenic PCR (PCR in the presence of manganese) of the region of the gene encoding the active site and **mutants** screened for increased efficiency of incorporation of a fluorescein deriv.-labeled base analog. Further characterization of the enzymes and their substrate utilization are reported.